

Review

Genetic Transformation of Forest Trees and Its Research Advances in Stress Tolerance

Yi Li ^{1,2,†}, Yanhui Yuan ^{1,2,†}, Zijian Hu ^{1,2}, Siying Liu ¹ and Xi Zhang ^{1,2,*} 

¹ State Key Laboratory of Tree Genetics and Breeding, State Key Laboratory of Efficient Production of Forest Resources, National Engineering Research Center of Tree Breeding and Ecological Restoration, College of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, China; liyi233@bjfu.edu.cn (Y.L.); yhyuan@bjfu.edu.cn (Y.Y.); huzijian@bjfu.edu.cn (Z.H.); lsy746911@bjfu.edu.cn (S.L.)

² Institute of Tree and Genome Editing, Beijing Forestry University, Beijing 100083, China

* Correspondence: zhangxi@bjfu.edu.cn

[†] These authors contributed equally to this work.

Abstract: Forests represent a vital natural resource and play a crucial role in climate regulation and maintaining biodiversity. However, the growth and development of forest trees are increasingly challenged by rising environmental pressures, particularly detrimental abiotic stressors. To address these challenges, genetic transformation technologies have emerged as effective solutions. Despite various difficulties in genetic transformation for forest trees, including prolonged life cycles, genetic diversity, interspecies variations, and complex regeneration systems, significant research progress has been achieved in tree gene editing, transgenic technology, and methods for delivering exogenous molecules. These technologies have the potential to enhance tree quality, increase productivity, and improve resistance to abiotic stress. This review provides an overview of the main methods and transformation receptors in tree genetic transformation. Additionally, we summarize several novel techniques, such as nanoparticle-mediated gene transformation, advanced gene editing technology, various novel delivery carriers, and non-genetically modified protein function interference through peptide aptamer. Notably, we also place emphasis on several referable genes from forest trees and common crops, together with their potential function for improving abiotic stress responses. Through this research, we aspire to achieve sustainable utilization and conservation of tree resources, thereby providing substantial support for future livelihoods and economic development.

Keywords: genetic transformation; transformation receptor; abiotic stress; nanoparticle; peptide aptamer



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1. Introduction

Forests represent a vital natural resource, with their yield and quality exerting a direct influence on human life quality and economic development [1,2]. Traditional breeding methods are inadequate to cope with escalating environmental pressures and the intricate challenges posed by pests and diseases. Moreover, the genetic improvement of trees is constrained by issues such as long-life cycles, long generation times, late sexual maturity and limited genetic diversity [3,4]. Therefore, tree genetic transformation has become a focal point of intensive research. This process involves leveraging modern biotechnological approaches to introduce exogenous genes or other genetic material into plant cells, thereby altering their genetic characteristics. Genome editing technology is a potent approach for modifying, adding, or deleting genes within the genome [5]. In recent years, cisgenics has emerged as a technology distinct from transgenics, as it excludes the introduction of genes from other species [6]. Occasionally, gene editing techniques are employed to introduce synthetic or artificially engineered genes into host cells [7]. These advancements have propelled significant research progress in fields such as agriculture, forestry, and environmental conservation, demonstrating vast application prospects [8,9].

Tree genetic transformation emerges as a potent method, yielding enhancements in stress tolerance, yield augmentation, and quality enhancement (Figure 1). For instance, the introduction of drought-tolerant genes allows trees to flourish in arid environments. Furthermore, altering tree growth patterns and lignin content can enhance salt tolerance [10]. This review commences with a concise overview of the methods and transformation receptors utilized in tree genetic transformation and then delves into recent advances in techniques promoting transformation efficiency and success rate. We systematically summarize numerous applications of genetic transformation in improving tree stress tolerance and highlight the potential application prospects and challenges of these technologies in enhancing stress tolerance.

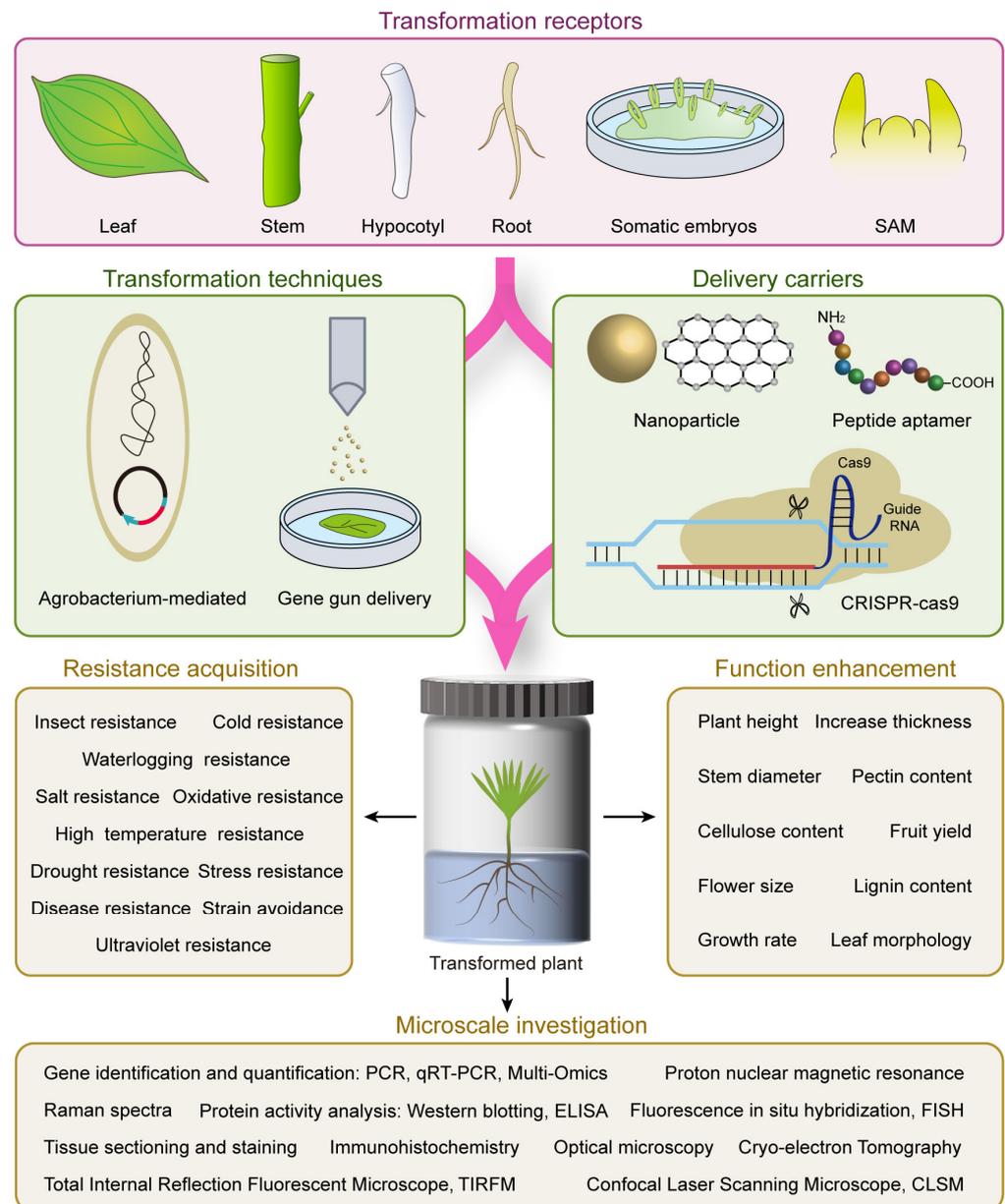


Figure 1. Ideograph showing common experimental procedure and transformation receptor used in forest tree genetic transformation and following investigation. The procedure mainly consists of three parts, including the selection and preparation of the transformation receptor, the utilization of appropriate transformation techniques to introduce the delivery carrier into the transformation receptor, and finally conducting stress resistance testing, functional analysis, and microscale investigations on the successfully transformed plants.

2. Tree Genetic Transformation Techniques and Transformation Receptor

With the development of molecular biology and genetic engineering techniques, tree genetic transformation techniques have significantly improved and become more refined. Currently, commonly used tree genetic transformation methods include the gene gun method and the *Agrobacterium*-mediated method [11,12]. The choice of transformation receptor is an important factor influencing plant genetic transformation. Transformation receptors are isolated from plant tissues that can be genetically modified, such as organs, cells or protoplasts. Under controlled artificial conditions, the cultivation of transformation recipients is performed to obtain regenerated transgenic whole plants or to implement the technology for producing economically valuable products [13]. Due to the challenges in establishing a regeneration system for trees, the options for suitable transformation receptors for tree genetic transformation are limited [14]. Typically, tree cells or tissues are cultured and regenerated in vitro using tissue culture and regeneration techniques to obtain transformation receptors [15].

2.1. Common Tree Genetic Transformation Techniques

The common genetic transformation systems can be categorized into two types: the gene gun and the *Agrobacterium*-mediated method. In the year 1994, *GUS* was successfully transferred into the anther-derived calluses of *Hevea brasiliensis* by gene gun [16]. The synthetic *CRY1Ac* from *Bacillus thuringiensis* has been used to transform *Pinus taeda* through a gene gun. These transgenic plants exhibit high levels of resistance against *Dendrolimus punctatus* Walker [17]. *Agrobacterium*-mediated transformation is the preferred method for genetic transformation in trees. *Agrobacterium* in soil infects wounded sites in many dicotyledonous and gymnosperm plants. It inserts T-DNA into the plant genome upon infiltrating cells, ensuring stable inheritance via meiotic division. This forms the theoretical basis for *Agrobacterium*-mediated plant genetic transformation [18]. *Agrobacterium tumefaciens* stands as one of the primary strains used in plant genetic transformation research. Transgenic plants were successfully generated in *Abies nephrolepis*, *Pinus elliotii* and *Pinus radiata* through the utilization of *Agrobacterium* strains including LBA4404, GV3101, and EHA105 [18–20]. *Agrobacterium rhizogenes* is another commonly used type of *Agrobacterium*. By using *A. rhizogenes* for tree genetic transformation, it facilitates gene function studies and rapid propagation of trees [21].

Selecting the appropriate *Agrobacterium* strain is essential in the genetic transformation of tree species, as it relies on their unique genetic traits, regenerative capabilities, and responses to different strains of *Agrobacterium* [22]. The leaf disc transformation mediated by *A. tumefaciens* was developed by Horsch et al. [23]. The genetic transformation of poplar leaves can be achieved with an efficiency exceeding 80% using the leaf disc [24]. However, based on our previous research, this method is challenging to implement in coniferous trees. Somatic embryogenesis can be employed for genetic transformation in deciduous pines such as *Pinus massoniana* and *Larix gmelinii*, but currently remains unattainable in *Pinus tabuliformis* [25]. We propose that the challenge of genetic transformation in *P. tabuliformis* could be addressed by selecting genotypes capable of somatic embryogenesis through screening. The choice of genetic transformation receptors plays a pivotal role in the success of plant genetic transformation.

2.2. Transformation Receptor Used in Forest Tree Genetic Transformation

Due to the intrinsic factors of trees and the difficulty in establishing regeneration systems, the transformation receptors suitable for tree genetic transformation primarily include mature and immature zygotic embryos, somatic embryos, seedlings, shoot meristems, and cotyledons [25–27]. Induced transformation receptors facilitate organ differentiation, leading to the development of shoots, roots, flowers, and the formation of a whole plant, typically categorized into indirect and direct organogenesis pathways [28]. Among these receptors, zygotic embryos and somatic embryos emerge as the most favorable for inducing embryogenic callus tissue [29]. Culturing both mature and immature embryos has

been demonstrated to enhance regeneration and genetic transformation capabilities [30]. The shoot apical meristem and leaves are common explants in trees [31]. Furthermore, although the root system of trees can also serve as an explant, its regeneration capacity is comparatively lower than that of shoot apical meristems and leaves, necessitating additional optimization conditions [32,33]. Cotyledons are also present as suitable receptors for genetic transformation in several tree species, such as pine and eucalyptus. For example, the well-developed cotyledons of *Pinus nigra* embryos were dissected into small pieces, and protoplasts were subsequently obtained through enzymatic digestion. Both the electroporation procedure and the particle bombardment procedure were employed to transform *uidA* into protoplasts, successfully enhancing the transient expression of *uidA* in *P. nigra* [34].

Somatic embryos are one of the most commonly used types of transformation receptors induced and cultured from mature tree tissues [35]. Utilizing GV3101 *Agrobacterium*-mediated RNAi, silencing of *PaWOX8/9* was conducted in embryos of *Norway spruce*, resulting in disrupted orientation of the cell division plane at the basal part, consequently leading to an aberrant morphology [36]. Moreover, within spruce species, zygotic embryos serve as another highly efficient conduit for genetic transformation [30]. Nonetheless, somatic embryos also pose some challenges, including instability during induction and culture processes, along with difficulties in obtaining high-quality embryos. Thus, the judicious selection of transformation receptors tailored to the specific traits of the target tree species and research objectives emerges as paramount in the realm of tree genetic transformation. The RAPID method capitalizes on plant regenerative capacities by injecting *A. tumefaciens* into meristematic tissues, inducing efficient transfection in newly formed tissues [37]. It outperforms traditional methods with increased transformation efficiency, a shorter duration, and the absence of tissue culture requirements. Consequently, this innovation overcomes limitations in achieving rapid plant transformation, showing promise for application in various plant species with active regeneration capabilities.

3. Advancements in Tree Genetic Transformation Process

The initiation of genetic transformation in forest trees dates back to 1988, with subsequent significant changes by the end of the 20th century [28]. Despite these advancements, several challenges still exist that hinder the effective implementation of genetic transformation in forest trees. One prominent challenge lies in the limited regenerative capacity observed in many forest tree species. Additionally, the long growth cycle of forest trees further complicates the timeline required for achieving mature transgenic individuals [4]. These factors contribute to the substantial temporal and financial investments associated with research in forest genetic transformation.

The cultivation of forest trees presents a range of challenges due to their varied tissue culture requirements, which necessitate the use of carefully optimized culture media compositions and cultivation conditions. Promising advancements in transformation technologies have led to significant progress, particularly when coupled with the development of forest embryos and the application of cutting-edge techniques [9]. Several recent reviews have explored the new technology of genetic transformation of forest trees in great detail [4,8,9]. Given the comprehensive coverage of these reviews, this section provides a concise overview focusing specifically on nanoparticle-mediated gene transformation, DNA-free gene editing technology and several novel delivery carriers.

3.1. Nanoparticle-Mediated Gene Transformation

With the continuous advancements in molecular biology and genetic engineering technologies, emerging gene delivery techniques have been introduced into tree genetic transformation. For example, methods like particle bombardment or electroporation enable the direct introduction of exogenous DNA into tree tissues, thereby improving the efficiency and stability of gene transformation [38,39]. In addition, a novel method utilizing nanoparticles (NPs) to facilitate gene transformation has emerged as a solution for the challenge posed by the plant cell wall [40]. By utilizing this approach, DNA or RNA molecules can

be precisely transferred into plants, resulting in either temporary or permanent genetic modifications [41]. For instance, LDH-DNA bioconjugates as sandwich nanostructures can efficiently carry DNA into the nucleus in BY-2 suspension cells serve novel molecular delivery systems [42]. In the future, with the target-specific delivery of NPs, the efficiency and success rate of genetic transformation in trees can be significantly improved.

3.2. Optimized Gene Editing Technologies

In pursuit of stable expression of exogenous genes and desired phenotypic changes, extensive work is conducted by researchers to optimize gene regulation. This includes selecting appropriate promoters and terminators, adjusting transgene copy numbers and insertion sites, and optimizing integration methods into the genome, ultimately improving exogenous gene expression and precise regulation of target genes in trees [43]. For example, the use of RNA interference and gene editing technologies, such as the CRISPR-Cas9 system, further optimize the selection and improvement of tree genetic materials [44]. These emerging technologies provide researchers with more possibilities for selecting and manipulating genetic materials. However, in woody species, except for poplar, the low transformation efficiency and in vitro regeneration capability, along with their inherently slow growth rate, pose significant bottlenecks for the more widespread implementation of genome editing technologies. In order to shorten the juvenile phase of woody plants and promote early flowering to ensure precocity, overexpression of the *BpMADS4* gene can be employed [45]. Flachowsky et al. reported that overexpression of *BpMADS4* in apple significantly reduced the juvenile phase and achieved early flowering [46]. Heterologous expression of FT from various donor species has been shown to shorten the generation time in *European plum*, *Eucalyptus*, *Populus*, and sweet orange [47].

The uORF in eukaryotic mRNA regulates translation by inhibiting the main coding open reading frame. CRISPR-Cas9 editing of uORF in rice mutants with altered traits offers a universal approach for predictable gene expression fine-tuning in molecular design breeding [48]. Artificial intelligence enhances large-scale protein structure prediction, with AI-assisted methods establishing a high-throughput clustering technique based on tertiary structures. This aids in exploring deaminase functional structures and identifying novel scaffold components [49]. Optimized gene editing methods increasingly serve as effective tools for tree genetic transformation and mitigating non-biological stressors.

3.3. DNA Free Gene Editing Technology

Traditional stable transformation, although widely used for genome editing in plants, requires considerable time and labor to generate DNA-free gene-edited crops through genetic segregation [50]. To improve efficiency, methods like fluorescent labeling and resistance screening are employed in CRISPR/Cas9 vectors [51]. Using microscopy, non-fluorescent transgenic materials are excluded, or resistance-sensitive methods are used to eliminate materials containing exogenous genes. The transgene killer CRISPR (TKC) system facilitates the self-elimination of CRISPR components, reducing time and labor for obtaining DNA-free plants with desired genomic modifications [52]. Through the gene gun method, CRISPR/Cas9 plasmid DNA (TECCDNA) or transcribed RNA (TECCRNA) is directly introduced into somatic cell embryos, where endogenous nucleases rapidly degrade the introduced DNA or RNA [53]. In contrast, RNA virus-mediated CRISPR/Cas9 gene editing ensures virus-free transgenic progeny plants, as RNA viruses cannot infect embryos or seeds, and their replication avoids integration into the host plant chromosomes [54]. This system offers an efficient and reliable tool for generating genetically modified forest trees with minimal foreign DNA content.

3.4. Peptide Aptamer Mediated Non-Genetically Modified for Protein Function Interference

Peptide aptamers are polypeptide chains composed of 8–20 amino acid residues that can specifically interact with target molecules. They can disrupt protein–protein interactions and deactivate the functionality of the target protein without altering the gene structure,

degrading mRNA, or modifying the protein structure [55]. Peptide aptamers have great potential applications in plant functional genomics as “suppressors” that bind to target proteins in plants and inhibit their functions. A 16-amino acid peptide aptamer library was screened with rice MAGO protein as bait, yielding the specific interacting aptamer, PAP [56]. PAP preferentially forms a disruptive heterodimer, PAP-MAGO, competing with MAGO-Y14 heterodimer formation and leading to phenotypic similarities between PAP-overexpressing rice plants and *OsMAGO*- and *OsY14*-RNAi plants. Moreover, the employment of *Agrobacterium*-mediated techniques for the delivery of viral vectors to plants, combined with the utilization of the amplification potential of RNA viruses, offers a promising avenue for the development of a “spray” technology capable of modifying crucial agronomic traits [57].

Peptide aptamers have marked potential in forest tree research, offering valuable applications in targeted control of precise regulation of desirable traits related to growth, wood quality, and stress resistance. Designing peptide aptamers enables modulation of gene expression, allowing tailored approaches to enhance tree characteristics [58]. Peptide aptamers can also enhance tree resilience to stress conditions such as drought and salinity by competitively inhibiting relevant molecular receptors, thereby effectively targeting and regulating tree responses. The peptide aptamer cPEP, specifically designed to target HSP101 in soybeans, exhibits a remarkable capability of enhancing their tolerance to heat stress [59]. Notably, peptide aptamers provide the advantage of internal molecule regulation in plants without altering the genome or resorting to genetic editing or engineering methods. Consequently, peptide aptamers can be externally applied through techniques like spraying or irrigation, facilitating trait improvement in tree species with limited genetic transformation systems.

4. Application of Genetic Transformation in Improving Tree Tolerance

As important components of ecosystems, enhancing the stress tolerance of trees is of significant importance for ecosystem stability and environmental protection. However, due to limitations imposed by natural selection and breeding processes, trees have relatively weak capabilities to withstand adverse conditions. Therefore, introducing stress-tolerance-related genes through tree genetic transformation has become an effective strategy to enhance tree stress tolerance [60]. In this section, we systematically summarized the application of genetic transformation in the fields of abiotic stress, biotic stress, and the accumulation of stress-resistant substances, while also discussing biosafety concerns associated with transgenic trees.

4.1. Application in Abiotic Stress

Abiotic stresses in plants include cold, freezing, drought, salt, nutrient deficiency, and heavy metals [61–64]. Through tree genetic transformation, genes related to abiotic stress can be introduced, enhancing the growth and survival capabilities of trees under these adverse conditions [65].

Genetic engineering of trees focuses on salt tolerance and drought tolerance. For example, salt stress-related genes discovered in salt-tolerant plants can be introduced into target tree species, improving their adaptability to saline soils. Heterologous expression of PeREM6.5 in *Populus euphratica* in *Arabidopsis* significantly increased the H⁺-ATPase hydrolytic activity and H⁺ transport activity in plasma membrane (PM) vesicles, thereby improving the plant's ability to maintain ion homeostasis under salinity [66]. The introduction of drought tolerance genes can reduce water loss and maintain better water balance in transgenic trees under drought conditions. Overexpression of *PeCHYR1* in poplar 84 K increased drought tolerance through ABA-induced hydrogen peroxide (H₂O₂) production, resulting in stomatal closure [67]. Furthermore, by introducing genes for cold tolerance or heavy metal resistance, the ability of trees to withstand low temperatures and tolerate soil environments polluted with heavy metals can be improved, mitigating the impacts of freezing weather and polluted soils [68].

The prominent function of lignin is to provide mechanical strength and rigidity to the cell wall, facilitating the formation of xylem vessels for long-distance transportation of water and nutrients [69]. In addition, lignin contributes to various abiotic stress responses in plants. Transcriptional upregulation of lignin biosynthesis genes such as *C4H*, *C3H*, *CAD*, *F5H*, *HCT*, *4CL*, *COMT*, *CCR*, and *CCoAOMT* leads to lignin deposition, secondary cell wall thickening, and enhanced salt tolerance and osmotic resistance in *Betula platyphylla* and *Malus domestica* [70,71]. We provide a detailed review of recent advances in abiotic stress using genetic transformation methods (Table 1).

Table 1. Genes and functions related to stress resistance in different tree species.

Stress	Tree Species	Gene	Transformation Receptor	Transformation Technique	Trait	Reference
Drought	<i>Betula platyphylla</i>	<i>Dof4</i> <i>Dof11</i> <i>Dof17</i>	Seedling	EHA105	Scavenging ROS Reduce cell damage	[72]
Drought	<i>Betula platyphylla</i>	<i>ERF2</i>	Seedling	EHA105	Scavenging ROS Keep cell wall integrity	[73]
Drought	<i>Betula platyphylla</i>	<i>MYB102</i>	Seedling	EHA105	Scavenging ROS	[73]
Drought	<i>Populus tremula</i> × <i>Populus alba</i>	<i>GS1a</i>	Leaf	LBA4404	Regulate abiotic stress Involved in plant nitrogen metabolism	[74]
Drought	<i>Populus trichocarpa</i>	<i>PYRL1</i> <i>PYRL5</i>	Leaf	EHA105	ABA receptor	[75]
Cold	<i>Picea wilsonii</i>	<i>NF-YB3</i>	Flower	GV3101	Modulate gene regulation in CBF-dependent pathway	[76]
Drought	<i>Picea wilsonii</i>	<i>NAC30</i>	Flower	GV3101	Regulate abiotic stress	[77]
Salt	<i>Populus euphratica</i>	<i>CBF4</i>	Leaf	GV3101	Increase photosynthetic rate High SOD activities	[78]
Drought	<i>Populus trichocarpa</i>	<i>NDPK2</i>	Leaf	LBA4404 EHA105	Increase expression of auxin-related indole acetic acid gene	[79]
Salt	<i>Pinus taeda</i>	<i>Mt1D</i> , <i>GutD</i>	mature zygotic embryo	LBA4404	Produce mannitol and glucitol	[80]
Salt	<i>Populus simonii</i> × <i>Populus nigra</i>	<i>ERF76</i>	Leaf	EHA105	Involved in ABA signal pathway	[81,82]
Salt	<i>Populus trichocarpa</i>	<i>CYP714A3</i>	Leaf	EHA105	Reduce GA synthesis Response to salt toxicity	[83]
Salt	<i>Populus deltoides</i> × <i>Populus nigra</i>	<i>PTP1</i>	Leaf	EHA105	Affect Na ⁺ /K ⁺ and ROS homeostasis	[84]
Salt	<i>Betula platyphylla</i>	<i>RAV1</i>	Seedling	EHA105	Scavenging ROS	[85]
Osmotic stress						
Stress response	<i>Litchi chinensis</i>	<i>MYB1</i>	Hairy root	MSU440	Anthocyanin biosynthesis	[86]
Abiotic stress	<i>Betula platyphylla</i>	<i>ERF98</i>	Leaf	GV3101	Improve the tolerance to abiotic stress	[87]

Plants have various mechanisms to cope with abiotic stress, including stress tolerance, avoidance, escape, and recovery mechanisms [88]. Upon recognizing stressful conditions, plant cells activate these responses to restore cellular and organismal homeostasis. These mechanisms can also mitigate the impact of chronic stress [89]. Some common characteristics of drought, salt, and cold stress responses include the initiation of intracellular Ca²⁺ spikes, physiological adaptations to water deficits, the accumulation of various osmolytes and antioxidants, changes in phospholipid composition, the generation of various reactive oxygen/nitrogen species, and the triggering of phosphorylation cascades [90]. These stresses can lead to irreversible changes, restricting cell division and growth, reducing fertility, promoting senescence, and even causing cell death under extreme conditions.

Over the past few decades, numerous genetically modified or transgenic tree varieties with enhanced traits and novel characteristics have been generated. These trees have been associated with widespread, yet unconfirmed, concerns regarding health and environmental safety. The analysis of the transgenic line *JERF36*, obtained 15 years ago, which enhanced salt tolerance in hybrid poplar (*P. alba* × *P. berolinensis*), revealed that a greater number of genes are influenced by environmental factors compared to those introduced by the *JERF36* gene. The synergistic impact of environmental factors and exogenous genes outweighs transgenesis and *JERF36* introduction effects [91]. In addition, a biosafety assessment of the herbicide-tolerant transgenic eucalyptus tree 751K032 was conducted in Brazil [92]. The study revealed that 751K032 is as safe for humans, animals, and the environment as the

traditional clone FGN-K. This genetic modification has enhanced productivity, making it a valuable and sustainable tool for wood production. Considering public concerns about biosafety issues associated with the introduction of exogenous genes into plants, cisgenesis involves only the genes of interest from the host plant and inserting them into the host genome without incorporating undesired genomic regions. For instance, in the context of introgression breeding programs, durable and resistant potato varieties have been developed by introducing three different *Phytophthora* sp. resistant genes from wild species, such as *Solanum demissum* and *S. bulbocastanum*, through cisgenic breeding in a relatively short period of time [7].

In the past few decades, the escalating global population, together with the surge of natural calamities, political unrest, and climate change, has aggravated the global food supply crisis. This critical situation has sparked extensive research into the adaptation of staple crops to abiotic stress, thereby yielding invaluable research references and genetic resources for targeted breeding and genetic modification in tree genetics. In the field of temperature stress research, studies have revealed the involvement of the quantitative trait locus gene *COLD1* in rice, which encodes a nine-transmembrane protein and interacts with *RGA1* to perceive low-temperature signals [93]. Overexpression of *COLD1* in both maize and grapes significantly enhances the plants' cold resistance [94]. Introducing *COLD1* expression in trees through genetic transformation could expand cultivation possibilities for various tree species. In addition, *OsTT* and *ERECTA* genes that are resistant to temperature stress were also found in rice and other crops [95]. In drought stress, receptor kinase *GHR1* has been found to coordinate the regulation of H_2O_2 and ABA signals to shut down guard cells [96]. Furthermore, research has demonstrated the interplay between the TOR and ABA signaling pathways in regulating the balance between plant drought tolerance and growth [97]. The mechanism analysis of abiotic stress resistance in these model plants has certain reference significance for trees to resist abiotic stress. We summarized the genes that have responded to abiotic stress in model plants and crop species in recent years, so as to provide inspiration for tree research (Table 2).

Table 2. Genes related to abiotic stress in common crop species.

Stress	Species	Gene	Transformation Receptor	Reference
Chilling	<i>Oryza sativa</i>	<i>COLD1</i>	Embryonic calli	[93]
Heat		<i>TT1</i>	Embryonic calli	[98]
Drought		<i>SNAC1</i>	Embryonic calli	[99]
Salt Drought		<i>bZIP23</i>	Embryonic calli	[100]
Heat		<i>TOGR1</i>	Embryonic calli	[101]
Chilling		<i>bHLH002/ICE1</i>	Embryonic calli	[102]
Salt	<i>Triticum aestivum</i>	<i>ERF3</i>	Embryonic calli	[103]
Drought		<i>SAP5</i>	Immature embryo	[104]
Drought		<i>WRKY1,</i> <i>WRKY33</i>	flower	[105]
Heat		<i>FER-5B</i>	Immature embryo	[106]
Heat		<i>bZIP60</i>	flower	[107]
Salt		<i>MYB32</i>	flower	[108]
Salt		<i>SRO1</i>	Shoot apical meristem	[109]
Salt		<i>OPR1</i>	Shoot apical meristem	[110]
Salt		<i>GCN5</i>	flower	[111]
Drought Heat		<i>DREB2A</i>	flower	[112]
Drought		<i>VPP1</i>	Immature embryo	[113]
Drought	<i>Zea mays</i>	<i>NAC111</i>	Immature embryo	[114]
Drought		<i>DREB2.7</i>	flower	[115]
Salt		<i>HKT1</i>	Immature embryo	[116]

4.2. Application in Production and Accumulation of Stress-Resistant Substances

By introducing relevant genes, trees can produce and accumulate stress-resistance substances when facing adverse conditions, thereby enhancing their resistance in stressful environments. On one hand, tree genetic transformation can introduce genes involved

in antioxidant synthesis, such as carotenoid synthesis genes and superoxide dismutase genes, to enhance the tree's resistance against oxidative stress. The expression of these genes can promote the production and accumulation of antioxidants, helping to remove free radicals within cells, slow down or prevent oxidative damage, and protect the stability and function of cells. Overexpressing *PagDA1a* or *PagDA1b* improved salt tolerance and drought resistance in transgenic poplar by optimizing ion homeostasis and enhancing active oxygen scavenging capacity [117]. Additionally, tree genetic transformation can introduce genes related to the accumulation of stress substances, such as proline synthesis genes. Proline is an important stress-resistant substance that has the ability to resist drought and low temperatures. For instance, drought stress produces the accumulation of free proline, which can enhance their tolerance to stress conditions [118].

4.3. Application in Biotic Stress

Diseases and pest infestations are significant factors that limit the healthy growth of trees. By introducing genes related to insect and disease resistance, trees can resist the invasion of pests and diseases, thereby improving their survival and disease resistance abilities. For example, by introducing genes with antiviral capabilities, the immune response of trees to viral infections can be enhanced. Hairpin-inducing silencing constructs based on *Prunus persica* orthologs in *Prunus salicina* can effectively silence *Plum pox virus* (PPV) infection caused by initiation factor eIFiso4G, and silenced plants exhibit persistent and stable resistance to PPV [119]. Genes for resistance against bacterial and fungal infections can also be introduced into target tree species using genetic transformation techniques to enhance their resistance against pathogenic microorganisms. For example, overexpressing *Arabidopsis AtGolS* and *Cucumber sativus CsRFS* in *Populus alba* × *P. grandidentata* challenged poplar leaf rust defense responses by inhibiting reactive oxygen species and attenuating calcium and phosphatidic acid signaling events leading to SA defense [120].

Additionally, the introduction of genes for insect resistance can enhance the ability of trees to withstand insect attacks. BxML1 is an effector of *Bursaphelenchus xylophilus*, which inhibits the immune response triggered by the BxCDP1 molecular pattern of *B. xylophilus*. When *BxML* was silenced, the number and incidence of *B. xylophilus* infected with *Pinus thunbergii* and the parasitism and virulence of *P. thunbergii* were reduced [121]. The Chinese pine is constantly under attack by *Bursaphelenchus xylophilus*, and there is currently no effective solution. Introducing exogenous genes that confer resistance to *Bursaphelenchus xylophilus* can help alleviate the economic losses caused by pest infestations in Chinese pine. Certain genes or transcription factors exhibit broad-spectrum disease resistance, and their disease resistance in trees can be enhanced through genetic transformation methods. The transformation of the *OxO* gene from wheat into the American chestnut leads to the degradation of oxalic acid secreted by *Cryphonectria parasitica*, reducing the fungus's virulence. qPCR results show a significant, approximately 200-fold, increase in *OxO* expression levels in the modified American chestnut [122].

5. Conclusions and Future Prospects

Tree breeding encompasses various techniques, including cuttage, graft, genetic transformation, etc. Genetic transformation of forest trees is a crucial and key technology that significantly contributes to the improvement of tree characteristics. These technologies play a vital role in enhancing the quality, increasing yield, and improving the stress tolerance of trees, particularly against abiotic stress. They provide an effective tool for breeding high-yielding, superior-quality, and stress-tolerant tree varieties while also enhancing their adaptability to abiotic stress, driving the development of agriculture and forestry, and promoting environmental conservation. However, the methods of genetic transfer and manipulation, such as transgenic and cisgenic approaches, are not entirely precise [6]. While scientists can control the trait genes (or their synthetically engineered equivalents) to be inserted into the host plant genome with relative precision, they cannot fully control their location or the number of copies inserted. Cisgenics manipulates only the genome

within a specific plant. Therefore, these techniques may have fewer concerns regarding pleiotropy. Nonetheless, ensuring stable and efficient genetic transformation remains a crucial issue in modern biology. The challenges come from the fact that trees are chimeras, with different parts undergoing development at varying times and stages, leading to inherent inefficiencies in the genetic transformation of trees.

In the future, further research and exploration are imperative to address the aforementioned technical challenges and establish the safety and feasibility of the practical application of this technology. Additionally, it is crucial to prioritize safety and ethical considerations when employing this technology for tree genetic transformation. This will contribute to ensuring the stability and sustainable development of the technology, making positive contributions to the conservation and utilization of future forest resources.

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